Rapid SARS-CoV-2 Antigen Test Card

Healthcare Provider Instructions for Use

For in vitro diagnostic use only
For use under an Emergency Use Authorization (EUA) only
For use with anterior nasal swabs specimens

1.1 Intended Use

The Rapid SARS-CoV-2 Antigen Test Card is a lateral flow immunoassay intended for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2. This test is authorized for non-prescription home use with self-collected anterior nasal (nares) swab samples from individuals aged 14 years or older or adult collected anterior nasal (nares) swab samples from individual aged two years or older. This test is authorized for individuals with symptoms of COVID-19 within the first 6 days of symptom onset when tested at least twice over three days with at least 48 hours between tests, and for individuals without symptoms or other epidemiological reasons to suspect COVID-19, when tested at least three times over five days with at least 48 hours between tests.

The Rapid SARS-CoV-2 Antigen Test Card does not differentiate between SARS-CoV and SARS-CoV-2.

Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen. Antigen is generally detectable in anterior nasal (nares) swabs during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient medical history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Individuals who test positive with the Rapid SARS-CoV-2 Antigen Test Card should self-isolate and seek follow up care with their physician or healthcare provider as additional testing may be necessary.

All negative results should be treated as presumptive and confirmation with a molecular assay, if necessary for patient management, may be performed. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control measures such as isolating from others and wearing masks.

Negative results should be considered in the context of an individual's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19.

Individuals who test negative and continue to experience COVID-like symptoms of fever, cough and/or shortness of breath may still have SARS-CoV-2 infection and should seek follow up care from their healthcare provider.

Individuals should provide all results obtained with this product to their healthcare provider for public health reporting. All healthcare providers will report all test results they receive from individuals who use the authorized product to relevant public health authorities in accordance with local, state, and federal requirements, using appropriate LOINC and SNOMED codes, as defined by the Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests provided by CDC.

The Rapid SARS-CoV-2 Antigen Test Card is authorized for non-prescription self-use and/or as applicable an adult lay user testing another person 2 years or older in a non-laboratory setting.

The Rapid SARS-CoV-2 Antigen Test Card is only for use under the Food and Drug Administration's Emergency Use Authorization. This product has not been FDA cleared or approved.

1.2 Explanation of the Test

COVID-19 (short for 'Coronavirus Disease 2019') is a disease first recognized in 2019 that is caused by a type of novel coronavirus called SARS-CoV-2. Due to its rapid spread, the World Health Organization (WHO) recognized the disease as a global pandemic on March 11, 2020. Individuals infected with SARS-CoV-2 may have a range of symptoms from asymptomatic infection to severe respiratory illness and even death. The virus is spread primarily from person to person through respiratory particles, even by individuals without symptoms.

The Rapid SARS-CoV-2 Antigen Test Card is a rapid, qualitative immunochromatographic assay for the determination of the presence of SARS-CoV-2 antigens in anterior nasal swab specimens. Rapid SARS-CoV-2 Antigen Test Card employs a double antibody sandwich method. Colloidal gold conjugated anti-SARS-CoV-2 antibodies are dry-immobilized on the test device. When the specimen is added, it migrates by capillary diffusion through the strip to re-hydrate the gold conjugate complexes. If present at or above the limit of detection, SARS-CoV-2 viral antigens will react with the gold conjugate complexes to form particles, which will continue to migrate along the strip until the Test Zone (T) where they are captured by the immobilized anti-SARS-CoV-2 antibodies to form a visible pink/purple line. If there are no SARS-CoV-2 viral antigens in the specimen, no pink/purple line will appear in the Test Zone (T). The gold conjugate complexes will continue to migrate until being captured by immobilized antibody in the Control Zone (C) to form a pink/purple line, which indicates adequate fluid transfer past the test line to the control line of the test.

1.3 Materials Provided

Components	1 Test per Box	2 Tests per Box	4 Tests per Box	5 Tests per Box	8 Tests per Box	10 Tests per Box	20 Tests per Box	40 Tests per Box
Rapid SARS-CoV-2 Antigen Test Card (sealed foil pouch)	1	2	4	5	8	10	20	40
Sterilized Swab	1	2	4	5	8	10	20	40
Extraction Buffer Tube	1	2	4	5	8	10	20	40
Tube Holder	1 (packaging)	1 (packaging)	1	1	2	2	5	5
Quick Reference Instructions	1	1	1	1	2	2	5	5

1.4 Materials Required but not Provided

Clock or timer

1.5 Quality Control

Each Rapid SARS-CoV-2 Antigen Test Card has a built-in internal procedural control. The pink/purple line appearing at the "C" position is an internal procedural control. This procedural control line indicates that sufficient flow has occurred, and the functional integrity of the test device has been maintained. A distinct reddish-pink Control line should always appear if the test has been performed correctly, independently of whether a test line appears or does not appear on the test. If the Control line does not appear, the test result is invalid and a new test should be performed.

External run controls are not required to use the Rapid SARS-CoV-2 Antigen Test Card in a home setting.

1.6 Test Procedures

1 

Wash your hands with soap and water, or use hand sanitizer, before performing the test.

2 Check test expiration date on the test cassette pouch.

3 Bring the kit to room temperature when you are ready to begin the test.

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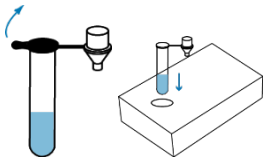

When you are ready to perform the test, remove the seal from the buffer tube and place the tube in the tube holder.

Open it away from your face and be careful not to spill any of the liquid.

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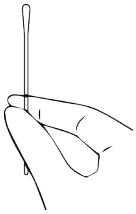

Peel open the swab packaging and gently take out the swab.

Be careful not to touch the soft, fabric tip of the swab.

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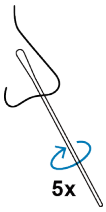

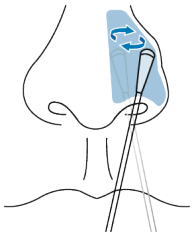

Holding the stick end of the swab, gently insert the entire absorbent tip of the swab into the nostril **no more than ½ to ¾ inch**. There is no need to go deeper.

Slowly rotate the swab in a circular motion **5 times** by firmly pressing against the inside walls of the nostril **for a total of 15 seconds. Do not just spin the swab.**

Gently remove the swab and repeat in the other nostril using the same swab.

WARNING: Inaccurate test results may occur if the nasal swab specimen is not properly collected.

When swabbing others, please wear a face mask. With children, you may not need to insert the swab as far into the nostril. For very young children, you may need another person to hold the child's head while swabbing.

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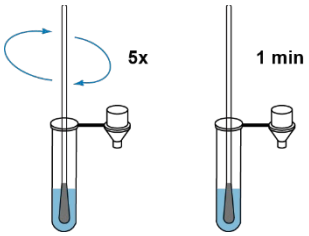
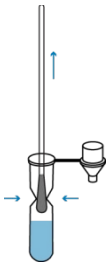


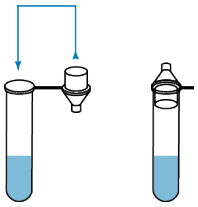
Place swab into buffer tube. **Rotate swab 5 times**.

Set a timer and leave swab in buffer tube for 1 minute.

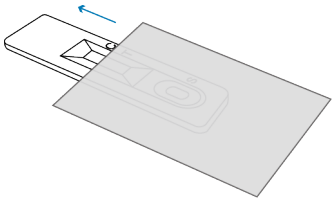
8 

Pinch buffer tube with fingers and remove the solution from swab as much as possible.

WARNING: Failure to squeeze the tube can lead to incorrect results due to excess buffer in the swab.

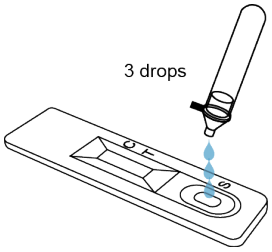
9 

Press the cap onto the buffer tube until it is secure.

10 

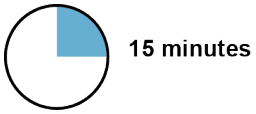
Open the pouch and remove the test cassette. Place the cassette on a flat and level surface.

WARNING: Once opened, the test cassette must be used within 30 minutes, otherwise inaccurate results may occur.

11 

Invert the buffer tube and add 3 drops of test sample into the sample well (S) by gently squeezing the extraction tube. **Do not add to the rectangular results window.**

WARNING: Adding other then the recommended number of drops may result in inaccurate results.

12 

Set a timer and read the results at 15 minutes.

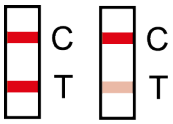
WARNING: Do not read the result before 15 minutes or after 30 minutes.

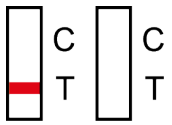
After test is completed, dispose of used materials in the trash.

1.7 Interpretation of Results

WARNING: Do not read the result before 15 minutes or after 30 minutes. Inaccurate test interpretations may occur.

Look at the result window and locate the letters C and T on the side of the window. A pink/purple line should always appear at the C position; this is a control line and signals that the test is working properly.

	**Negative result** If the Control (C) line is visible, but the Test (T) line is not visible, the test is negative. **To increase the chance that the negative result for COVID-19 is accurate, you should:** **•Test again in 48 hours if the individual has symptoms on the first day of testing.** **•Test 2 more times at least 48 hours apart if the individual does not have symptoms on the first day of testing.** A negative test result indicates that the virus that causes COVID-19 was not detected in the sample. A negative result does not rule out COVID-19. There is a higher chance of false negative results with antigen tests compared to laboratory-based tests such as PCR tests. If the test is negative but COVID-19-like symptoms, e.g., fever, cough, and/or shortness of breath continue, follow up testing for SARS-CoV-2 with a molecular test or testing for other respiratory disease should be considered. If applicable, seek follow up care with the primary health care provider. All negative results should be treated as presumptive and confirmation with a molecular assay may be necessary if there is a high likelihood of SARS-CoV-2 infection, such as in an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions.
	**Positive result** If the Control (C) line and the Test (T) line are visible, the test is positive. Any faint visible test (T) line with the control line (C) should be read as positive. **Repeat testing does not need to be performed if patients have a positive result at any time.** A positive test result means that the virus that causes COVID-19 was detected in the sample, and it is very likely the individual has COVID-19 and is contagious. Please contact the patient's doctor/primary care physician (if applicable) and the local health authority immediately and instruct your patient to adhere to the local guidelines regarding self-isolation. There is a very small chance that this test can give a positive result that is incorrect (a false positive). Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Individuals who test positive with the Rapid SARS-CoV-2 Antigen Test Card should self-isolate and seek follow up care with their physician or healthcare provider as additional confirmatory testing with a molecular test for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposures to COVID-19 or residing in communities with low prevalence of infection.

	**Invalid result** If the control (C) line is not visible, the test is invalid. Re-test with a new swab and new test device.

Repeat testing is needed to improve test accuracy. Please follow the table below when interpreting test results.

Status on First Day of Testing	First Result Day 1	Second Result Day 3	Third Result Day 5	Interpretation
With Symptoms	Positive	N/A	N/A	Positive for COVID-19
	Negative	Positive	N/A	Positive for COVID-19
	Negative	Negative	N/A	Negative for COVID-19
Without Symptoms	Positive	N/A	N/A	Positive for COVID-19
	Negative	Positive	N/A	Positive for COVID-19
	Negative	Negative	Positive	Positive for COVID-19
	Negative	Negative	Negative	Negative for COVID-19

Results should be considered in the context of an individual's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19.

To report your test result, please go to www.bosoncov.com and follow the instructions.

1.8 Storage and Stability

- Rapid SARS-CoV-2 Antigen Test Card should be stored between 2 to 30 °C (35.6 to 86 °F).
- Kit components in the Rapid SARS-CoV-2 Antigen Test Card are stable until the expiration date printed on the label.
- The Test Device must remain in the sealed foil pouch until use.
- The shelf-life of the Rapid SARS-CoV-2 Antigen Test Card is 6 months and it is stable until the expiration date marked on the packaging.

1.9 Warnings and Precautions

- Read all instructions carefully before performing the test. Failure to follow the instructions may result in inaccurate test results.
- In the USA, this product has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization. This product has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

- **Serial testing should be performed in individuals with negative results at least twice over three days (with 48 hours between tests) for symptomatic individuals and three times over five days (with at least 48 hours between tests) for asymptomatic individuals. You may need to purchase additional tests to perform this serial (repeat) testing.**
- If you have had symptoms longer than 6 days you should consider testing at least three times over five days with at least 48 hours between tests.
- An anterior nasal swab sample can be self-collected by an individual age 14 years and older. Children age 2 to 13 years should be tested by an adult.
- Do not use on anyone under 2 years of age.
- Wear a safety mask or other face-covering when collecting a specimen from a child or another individual.
- Do not use if any of the test kit contents or packaging is damaged.
- Test components are single-use. Do not re-use.
- Do not use kit past its expiration date. For most current expiration dates of this test please refer to: http://www.fda.gov/covid-tests.
- Do not touch the swab tip.
- Once opened, the test card should be used within 30 minutes.
- **Do not read test results before 15 minutes or after30 minutes. Results read before 15 minutes or after 30 minutes may lead to a false positive, false negative, or invalid result.**
- Avoid exposure of your skin, eyes, nose, or mouth to the solution in the tube.
- The chemicals in the reagent solution are hazardous to the skin and eye. Please see the below table for safety recommendations for skin and eye irritation.
- **Keep testing kit and kit components away from children and pets before and after use. Avoid contact with your skin, eyes, nose, or mouth. Do not ingest any kit components. The reagent solution contains harmful chemicals (see table below). If the solution contacts your skin, eyes, nose, or mouth, flush with large amounts of water. If irritation persists, seek medical advice: https://www.poisonhelp.org or 1-800-222-1222.**

Hazard Category (mixture)	GHS Hazard Class for mixture	Labeling of Harm(s)	Hazardous Ingredients (%)	Recommended PPE Statement
Category 2/2A	Eye Irritation	Causes serious eye irritation (H319)	Sodium chloride 7647-14-5/1% TERGITOL 15-S-9/1%	Wear eye protection
Category 3	Skin Irritation	Causes mild skin irritation (H316)	TERGITOL 15-S-9/1%	NA

- For more information on EUAs please visit: https://www.fda.gov/emergencypreparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergencyuse-authorization
- For the most up to date information on COVID-19, please visit: www.cdc.gov/COVID19

1.10 Limitations

- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between January 2022 and February 2022. The clinical performance has not been established for all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- There is a higher chance of false negative results with antigen tests than with laboratory-based molecular tests due to the sensitivity of the test technology. This means that there is a higher chance this test will give a false negative result in an individual with COVID-19 as compared to a molecular test, especially in samples with low viral load.
- All COVID-19 antigen test negative results are presumptive and confirmation with a molecular assay may be necessary.
- If the patient continues to have symptoms of COVID-19, and both the patient's first and second tests are negative, the patient may not have COVID-19, however additional follow-up may be needed.
- If the test is positive, then proteins from the virus that causes COVID-19 have been found in the sample and the individual likely has COVID-19.
- This test is read visually and has not been validated for use by those with impaired vision or color-impaired vision.
- Incorrect test results may occur if a specimen is incorrectly collected or handled.
- This test detects both viable (live) and nonviable SARS-CoV-2. Test performance depends on the amount of virus (antigens) in the sample and may or may not correlate with viral culture results performed on the same sample.

2 Performance Characteristics

2.1 Analytical Sensitivity: Limit of Detection (LoD)

The Limit of Detection (LoD) of the Rapid SARS-CoV-2 Antigen Test Card was determined using serial dilutions of the gamma irradiated inactivated SARS-CoV-2 (USA-WA1/2020). Contrived samples were prepared by spiking the strain into the pooled negative nasal wash obtained from healthy volunteers confirmed negative by RT-PCR. 50 µL virus solution were spiked onto the swabs that were then processed per IFU. The preliminary LoD initially determined by testing two-fold serial dilution series of 3 replicates was confirmed by testing in 20 replicates. The confirmed LoD for the Rapid SARS-CoV-2 Antigen Test Card was 1.4×10^2 $TCID_{50}$/mL. Based upon the testing procedure for this study the LoD of 1.4×10^2 $TCID_{50}$/mL equates to 7.0 $TCID_{50}$/swab.

The performance of this test device in the detection of the Omicron variant of SARS-CoV-2 was evaluated in a dilution series of clinical specimens which were positive for the Omicron variant. This testing was conducted by the National Institutes of Health (NIH) as a component of the Rapid Acceleration of Diagnostics (RADx®) initiative. The clinical specimens used to prepare this dilution series were not identical to the previous specimen

pools prepared and tested by RADx to assess performance with the omicron variant. Results from this dilution series cannot be compared to other specimen pools and do not indicate that a test will have different clinical performance compared to other EUA authorized tests. Compared to an EUA authorized RT-PCR method, the Rapid SARS-CoV-2 Antigen Test Card detected 100% of live virus Omicron samples at a Ct-value of 24.8 (n=5). Testing was also compared to two additional EUA-authorized OTC antigen tests (Assay #1 and Assay #2). Omicron dilutions at lower viral concentrations (Ct-values greater than 25.8) were not detected by the Rapid SARS-CoV-2 Antigen Test Card in this study.

Omicron Pool 2 – Live Omicron Clinical Samples	**Average N2 Ct (n=9)**	**Assay #1** **Percent Positive (n=5)**	**Assay #2** **Percent Positive (n=5)**	**Rapid SARS-CoV-2 Antigen Test Card Percent Positive (n=5)**
Omicron-Dilution 1	19.8	100	100	100
Omicron-Dilution 2	20.8	100	100	100
Omicron-Dilution 3	21.5	100	100	100
Omicron-Dilution 4	22.7	100	100	100
Omicron-Dilution 5	23.6	100	0	100
Omicron-Dilution 6	24.0	60	0	100
Omicron-Dilution 7	24.8	0	0	100
Omicron-Dilution 8	25.8	0	0	0
Omicron-Dilution 9	27.4	0	0	0
Omicron-Dilution 10	28.1	0	0	0
Omicron-Dilution 11	29.1	0	0	0

2.2 High-dose hook effect

The Rapid SARS-CoV-2 Antigen Test Card was tested with up to 2.8×10^5 $TCID_{50}$/mL of gamma irradiated inactivated SARS-CoV-2 (USA-WA1/2020) and no high-dose hook effect was observed.

2.3 Endogenous Interfering Substances

The Rapid SARS-CoV-2 Antigen Test Card was evaluated for performance in the presence of potentially interfering substances that may be found in a respiratory specimen. The

positive (3x LoD SARS-CoV-2) and negative specimens were tested with the addition of potentially interfering substances. The performance of Rapid SARS-CoV-2 Antigen Test Card was not affected by any of the potentially interfering substances listed in the table below at the concentrations tested.

Potentially Interfering Substance	Concentration Tested	Potentially Interfering Substance	Concentration Tested
Human Whole Blood (EDTA tube)	4% v/v	Mupirocin	10 mg/mL
Mucin (porcine stomach, type II)	0.5%	Tamiflu (Oseltamivir Phosphate)	5 mg/mL
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	Fluticasone Propionate	5% v/v
Naso GEL (NeilMed)	5% v/v	Body & Hand lotion (Cerave)	0.5%w/v
Nasal Drops (Phenylephrine)	15% v/v	Body Lotion with 1.2% dimethicone	0.5%w/v
Nasal Spray (Oxymetazoline)	15% v/v	Hand Lotion (Eucerin)	5% w/v
Nasal Spray (Cromolyn)	15% v/v	Hand Sanitizer with Aloe, 62% ethyl alcohol	5% v/v
Zicam	5% v/v	Hand Sanitizer cream lotion (vaseline)	15% v/v
Homeopathic (Alkalol)	10% v/v	Hand Sanitizer, 80% ethanol, fast drying	15% v/v
Sore Throat Phenol Spray	15% v/v	Hand Soap liquid gel (soft soap)	10% w/v
Tobramycin	4 µg/mL		

2.4 Analytical Specificity: Cross-reactivity and Microbial interference

Cross-reactivity and interference studies were performed for related pathogens, high prevalence disease agents, and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen of the nasal cavity. Each organism and virus (13 bacteria and 16 viruses) was tested in both the absence and presence of gamma irradiated inactivated SARS-CoV-2 (SARS-CoV-2 isolate USA-WA1/2020) at 3x LoD. All testing samples were prepared in the pooled negative nasal wash (PNW). No cross reactivity or interference was observed for any of the organisms tested, except for SARS-coronavirus which exhibited cross-reactivity when tested as 7.9×10^3 $TCID_{50}$/mL. A titration of SARS-CoV was performed to find the concentration at which cross-reactivity was no longer observed. Cross reactivity was no longer observed for SARS-CoV at 7.9×10^0 $TCID_{50}$/mL. These results are not unexpected in that the Rapid SARS-CoV-2 Antigen Test Card targets the nucleocapsid protein which is present on both SARS-CoV and SARS-CoV-2 viruses.

Microorganism	Concentration	Microorganism	Concentration
Human coronavirus 229E	1.43×10^5 $TCID_{50}$/mL	Rhinovirus	1.43×10^5 $TCID_{50}$/mL
Human coronavirus OC43	8.50×10^4 $TCID_{50}$/mL	*Haemophilus influenzae*	1.0×10^6 cfu/mL
Human coronavirus NL63	5.85×10^4 $TCID_{50}$/mL	*Streptococcus pneumonia*	1.0×10^6 cfu/mL
SARS-coronavirus*	7.9×10^0 $TCID_{50}$/mL	*Streptococcus pyogenes*	1.0×10^6 cfu/mL
MERS-coronavirus	1.0×10^6 $TCID_{50}$/mL	*Candida albicans*	1.0×10^6 cfu/mL
Adenovirus	1.43×10^5 $TCID_{50}$/mL	*Bordetella pertussis*	5.0×10^3 cfu/mL
Human metapneumovirus 4 Type B2	1.43×10^5 $TCID_{50}$/mL	*Mycoplasma pneumonia*	1.0×10^6 cfu/mL
Parainfluenza virus 1	1.43×10^5 $TCID_{50}$/mL	*Chlamydia pneumoniae*	1×10^6 ifu/mL
Parainfluenza virus 2	1.43×10^5 $TCID_{50}$/mL	*Legionella pneumophila*	1.0×10^6 cfu/mL
Parainfluenza virus 3	1.43×10^5 $TCID_{50}$/mL	*Mycobacterium tuberculosis*	1.0×10^6 cfu/mL
Parainfluenza virus 4b	1.43×10^5 $TCID_{50}$/mL	*Pneumocystis carinii*	1.0×10^6 nuclei/mL
Influenza A	1.43×10^5 $CEID_{50}$/mL	*P. jiroveci-S. cerevisiae*	1.0×10^6 cfu/mL
Influenza B	1.43×10^5 $CEID_{50}$/mL	*Staphylococcus aureus* subsp. *Aureus*	1.0×10^6 cfu/mL
Enterovirus 68	1.43×10^5 $TCID_{50}$/mL	*Staphylococcus epidermidis*	1.0×10^6 cfu/mL
Respiratory syncytial virus	1.0×10^5 pfu/mL	Pooled Negative Matrix	N/A

*Cross reactivity was observed when testing SARS-coronavirus concentrations at 7.9×10^3, 7.9×10^2, and 7.9×10^1 $TCID_{50}$/mL.

To estimate the likelihood of cross-reactivity with SARS-CoV-2 of organisms that were not available for wet testing, *in-silico* analysis using the Basic Local Alignment Search Tool (BLAST) managed by the National Center for Biotechnology Information (NCBI) was used to assess the degree of protein sequence homology. HKU1 nucleocapsid phosphoproteins was analyzed and the results are below.

- The homology between SARS-CoV-2 nucleocapsid protein and human coronavirus HKU1 nucleocapsid phosphoproteins is relatively low, 36.74% across 82% of sequences, but cross-reactivity cannot be ruled out.

2.5 Flex Study

A robust use of Rapid SARS-CoV-2 Antigen Test Card was demonstrated by seven (7) flex studies as follows;

1) Non-level positioning of Test Device
2) Varying the swab rotation number
3) Sample volume variability
4) Result reading time variability
5) Temperature and humidity

6) Lighting conditions
7) Disturbance while testing

2.6 Clinical Evaluation

A prospective study was completed at three (3) sites in the United States for clinical validation of the Boson Rapid SARS-CoV-2 Antigen Test Card in subject-collected anterior nasal (AN) swab samples. The study evaluated the investigational test's performance in symptomatic individuals (those suspected of COVID-19). A total of 186 symptomatic subjects were enrolled and each were currently experiencing symptoms associated with COVID-19, **within 6 days of symptom onset**. Each enrolled subject either self-collected one sample from their anterior nasal passages (from both nostrils), or had one sample collected from him/her by another individual. Each subject then had a mid-turbinate nasal swab sample collected from him/her by one of the study personnel. Test results from the Boson Rapid SARS-CoV-2 Antigen Test Card (candidate test) were compared to highly sensitive molecular FDA EUA Authorized SARS-CoV-2 assays to determine test performance. An analysis was performed which identified that 21/72 (29%) of study subjects had low viral loads based on the Ct values from a comparator method RT-PCR test. This may be associated with the Omicron variant since the low positive percentage in this study is higher than that observed in prior clinical studies for previously authorized COVID-19 rapid antigen tests. Antigen test performance decreases as the percent of low positives increases since the comparator method is significantly more sensitive than the candidate test. Therefore, to be consistent with previous studies, the analysis for the primary performance calculation was conducted to reflect a study population with 10-20% low positives. Multiple Percent Positive Agreement (PPA) are presented below for the positive samples cohort when a range of low positive samples was included (10% to 20%). At 10% low positives, the PPA was 82.7% and the negative percent agreement (NPA) was 99% with 95% confidence interval bounds of 71.1% to 90.4% for PPA and 95.2% to 99.6% for NPA respectively. This was the basis of the authorization. At 20% low positives, the PPA was 73.8% with 95% confidence interval bounds of 62.0% to 83.0%.

Primary Analysis					
	10% Low Positive	**12.5% Low Positive**	**15% Low Positive**	**17.5% Low Positive**	**20% Low Positive**
High Positive Samples	52	52	52	52	52
Low Positive Samples	6	8	10	12	13
Total Comparator Positive for PPA Calculation	58	60	62	64	65
Total Test Positives for PPA Calculation	48	48	48	48	48
PPA (%)	82.7%	80.0%	77.4%	75.0%	73.8%
95% CI (XX% - XX%)	71.1% - 90.4%	68.2% - 88.2%	65.6% - 86.0%	63.2%- 84.0%	62.0%- 83.0%
NPA (%)	99.1% (112/113)				
95% CI (XX%-XX%)	95.2%-99.8%				

When all study participants are included, the PPA is 67.1% and the NPA is 99.1% with the 95% confidence interval bounds of 55.7% to 76.8% for the PPA and 95.2% to 99.8% for the NPA, respectively.

Age Distribution and Positive Rate for Symptomatic Subjects within First 6 Days of Symptom Onset			
	Positivity Rate		
Age Group	**Number of Specimens**	**Number of Positives**	**Positivity Rate**
2 to 13 years	42	11	26.2%
14 to 24 years	35	8	22.9%
25 to 64 Years	91	26	28.6%
65 Years and older	18	5	27.8%
Total	186	50	26.9%

Percent Agreement of the Boson Rapid SARS-CoV-2 Antigen Test Card vs Composite Comparator by Cumulative Symptoms Onset Day				
Days of COVID-19 Symptoms	**Number of Specimens Tested**	**Boson Positives (TP)**	**Composite Comparator Positives**	**Percent Agreement**
Day 0	13	0	6	PPA: 0%
Day 1	55	16	22	PPA: 72.7%
Day 2	54	18	22	PPA: 81.8%
Day 3	27	9	10	PPA: 90%
Day 4	17	3	7	PPA: 42.9%
Day 5	10	2	3	PPA: 66.7%
Day 6	10	1	3	PPA: 33.3%
Total	186	49	73	PPA: 67.1%

Clinical Study with Serial Testing

A prospective clinical study was conducted between January 2021 and May 2022 as a component of the Rapid Acceleration of Diagnostics (RADx) initiative from the National Institutes of Health (NIH). A total of 7,361 individuals were enrolled via a decentralized clinical study design, with a broad geographical representation of the United States. Per inclusion criteria, all individuals were asymptomatic upon enrollment in the study and at least 14 days prior to it and did not have a SARS-CoV-2 infection in the three months prior to enrollment. Participants were assigned to one of three EUA authorized SARS-CoV-2 OTC rapid antigen tests to conduct serial testing (every 48 hours) for 15 days. If an antigen test was positive, the serial-antigen testing result is considered positive.

At each rapid antigen testing time point, study subjects also collected a nasal swab for comparator testing using a home collection kit (using a 15-minute normalization window between swabs). SARS-CoV-2 infection status was determined by a composite comparator method on the day of the first antigen test, using at least two highly sensitive EUA RT-PCRs. If results of the first two molecular test were discordant a third highly sensitive EUA RT-PCR test was performed, and the final test result was based upon the majority rule.

Study participants reported symptom status throughout the study using the MyDataHelps app. Two-day serial antigen testing is defined as performing two antigen tests 36 – 48 hours apart. Three-day serial antigen testing is defined as performing three antigen tests over five days with at least 48 hours between each test.

Out of the 7,361 participants enrolled in the study, 5,609 were eligible for analysis. Among eligible participants, 154 tested positive for SARS-CoV-2 infection based on RTPCR, of which 97 (62%) were asymptomatic on the first day of their infection, whereas 57 (39%) reported symptoms on the first day of infection. Pre-symptomatic subjects were included in the positive percent agreement (PPA) of asymptomatic individuals, if they were asymptomatic on the first day of antigen testing, regardless of whether they developed symptoms at any time after the first day of testing.

Performance of the antigen test with serial testing in individuals is described in Table below.

Data establishing PPA of COVID-19 antigen serial testing compared to the molecular comparator single day testing throughout the course of infection with serial testing. Data is from all antigen tests in study combined.

DAYS AFTER FIRST PCR POSITIVE TEST RESULT	ASYMPTOMATIC ON FIRST DAY OF TESTING			SYMPTOMATIC ON FIRST DAY OF TESTING		
	Ag Positive / PCR Positive (Antigen Test Performance % PPA)					
	1 Test	2 Tests	3 Tests	1 Test	2 Tests	3 Tests
0	9/97 (9.3%)	35/89 (39.3%)	44/78 (56.4%)	34/57 (59.6%)	47/51 (92.2%)	44/47 (93.6%)
2	17/34 (50.0%)	23/34 (67.6%)	25/32 (78.1%)	58/62 (93.5%)	59/60 (98.3%)	43/43 (100%)
4	16/21 (76.2%)	15/20 (75.0%)	13/15 (86.7%)	55/58 (94.8%)	53/54 (98.1%)	39/40 (97.5%)
6	20/28 (71.4%)	21/27 (77.8%)	16/18 (88.9%)	27/34 (79.4%)	26/33 (78.8%)	22/27 (81.5%)
8	13/23 (56.5%)	13/22 (59.1%)	4/11 (36.4%)	12/17 (70.6%)	12/17 (70.6%)	7/11 (63.6%)
10	5/9 (55.6%)	5/8 (62.5%)		4/9 (44.4%)	3/7 (42.9%)	

1 Test = one (1) test performed on the noted days after first PCR positive test result. Day 0 is the first day of documented infection with SARS-CoV-2.

2 Tests = two (2) tests performed an average of 48 hours apart. The first test performed on the indicated day and the second test performed 48 hours later.

3 Tests = three (3) tests performance an average of 48 hours apart. The first test performed on the indicated day, the second test performed 48 hours later, and a final test performed 48 hours after the second test.

3.1 Technical Support

For questions, or to report a problem, please call Technical Support at +1-800-689-7794 (Available Hours: Mon. to Fri.: 9 a.m. – 5 p.m. PST) or support@bosoncovt.com.

Test system problems may also be reported to the FDA using the MedWatch reporting system (phone: 1-800 FDA-1088; fax: 1-800 FDA-1078: or http://www.fda.gov/medwatch).

3.2 Ordering and Contact Information

Xiamen Boson Biotech Co., Ltd.
Tel: +1-800-689-7794
Email: info@bosoncovt.com

3.3 International Symbol Usage

You may see one or more of these symbols on the labelling/packaging of this product:

	Use-by date		Batch code		*In vitro* diagnostic medical device
	Catalog number		Consult instructions for use		Manufacturer
	Contains sufficient for <n> tests		Temperature limit		Do not reuse

 **Manufacturer:** Xiamen Boson Biotech Co., Ltd.
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Number: 082380

Effective Date: 2022-12-12